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REGULATIVE FACTORS IN THE ORDERLY GROWTH OF RETINO-TECTAL CONNEXIONS

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As a model for the study of the developmental patterning of brain pathways and connexions, the optic tract of lower vertebrates connecting the retina of the eye to the midbrain tectum offers a number of long-recognized advantages. Much of our current understanding of the developmental organization of brain circuits stems from experiments on this fairly simple and accessible optic system. There is good reason to think that the basic developmental mechanisms found to operate in the prefunctional shaping of this system for visual behaviour have wide application to the developmental patterning of the nervous system in general.

Strange as it may seem today, the first experiments on the growth of behavioural organization in this system were designed to determine whether the basic visual properties, like perception of directionality, size, movement, contour, and so on, are installed directly by the growth process itself or have to be organized through use and behavioural adjustment (Sperry, 1965). This was a little over 25 years ago now, when it was still believed that the growth of nerve fibres was non-selective, subject only to mechanical guidance. Chemical and electrical guidance appeared to have been ruled out, and several lines of evidence seemed to show that selective synaptic connexions are unimportant, at least for orderly behaviour. "Instinct" was still a bad word in science and the whole concept of the inheritance of behavioural patterns remained anathema to prevailing doctrines in psychology. No developmental machinery was then known or available, even in theory, by which the highly ordered and precisely designed neural connexions for behaviour could be "grown into" the nervous system without the aid of function. Neurobiotaxis, with its dependence on function for selectivity, was still the prevailing favourite among explanatory concepts. Even the old master, Cajal, had been willing

to leave it to function when it came to the detailed central adjustments of synaptic associations for behaviour.

We have come a long way since then, of course, and the issue of growth versus function seems now to be quite settled and dead, at least in the case of retino-tectal connexions. The question is nevertheless of some current concern with respect to many of the other fibre tracts of the brain, particularly in man. It would seem fairly safe to speculate in this regard that the patterning of most of the long fibre systems of the CNS, even in man, is primarily a problem of developmental mechanics, not one of learning. Another way of saying the same thing is that, if learning involves changes in the morphology of nerve connexions at all, these would appear to be confined to dendritic relations and local neuron circuits without affecting very much the patterning of the long fibre systems of the brain.

Once the growth-versus-function issue was settled in favour of growth (Sperry, 1944), attention was turned to the nature of possible developmental mechanisms. The next general question—and one still pertinent and basic to the nervous system as a whole—concerned the extent to which orderly selective function is dependent on selectivity and orderliness in the underlying neural connexions. As late as 1954 it was being suggested that orderly visual recovery might be explained even though the divided optic fibres reconnected in a random diffuse pattern, with retino-tectal communication based on a physiological coding–decoding scheme. Similarly the pre–1940 Resonance Principle of Weiss (1937) and a more recent interpretation along similar lines proposed by Szentágothai (1961) and Székely (1966) would provide for selective communication regardless of absence of selectivity in the morphology of the fibre connexions.

Evidence that the normal topographical projection on the tectum is in fact restored after section and scrambling of the optic fibres came first from experiments in which localized lesions were made in the frog tectum. The position and size of the resultant scotoma or blind area were found to be the same whether the localized lesion was made before division of the optic nerve or after regeneration (Sperry, 1944)—a result obtainable only if the regenerated projection map had been restored in its original pattern. Further confirmation on a more refined scale has since come from electrical mapping methods, particularly in the studies of Gaze and his associates (reviewed in Gaze, 1967). Confirmation has also been obtained with histological procedures (Attardi and Sperry, 1960, 1963). The patterning of the optic fibre pathways and their terminal connexions was shown in the latter studies to be selectively predetermined according to the locus of origin of the optic fibre in the retinal field. Fibres from different retinal

sectors were found to exhibit different growth patterns, like different species of plants, by which they selectively regained their various predetermined target zones in the tectal field.

Other experiments, with H. L. Arora and A. J. Limpo (see Sperry, 1965), have shown that colour perception—like position, directionality, movement, size, and contour perception—undergoes an orderly reinstatement in optic nerve regeneration. In summary, all the basic functional attributes of vision appear thus to be restored in regeneration of the scrambled optic fibres. Endogenous physiological properties of different sensory and neural cell types were presumed to be involved, as well as synaptic connexions. Where the experiments have been extended into prefunctional stages involving initial nerve outgrowth, similar results have been obtained (see Székely, 1966).

As early as 1942 it was inferred (Sperry, 1942) that optic fibres arising from different retinal loci must be distinguished from one another according to the location of their ganglion cell bodies in the retinal field, "probably through differential physical-chemical properties induced in them by differentiation of the optic cup in development". It was suggested further (Sperry, 1945) that the retina must undergo a gradient or field type of differentiation along at least two separate axes and that contralateral eye transplants might demonstrate that the anteroposterior gradient is established before the dorsoventral gradient. A corresponding biochemical specification of the tectal field was likewise inferred and it was postulated that differential affinities must exist between the tectal neurons and the optic fibres. With supporting results from the vestibular, cutaneous, central and motor systems (Sperry, 1951*a*, *b*) the basic concepts were extended to the development of the nervous system generally. This material has been reviewed in some detail on four separate occasions in the past three years (Gaze, 1967; Jacobson, 1966; Sperry, 1965; Székely, 1966) and does not need further amplification.

Recent work on the optic system has been aimed at more detailed analysis of the various phases of the postulated specification processes, chemical-affinity mechanisms, and related factors. Gaze, Jacobson and Székely (1963) tested the elasticity of the tectal gradients in response to ingrowth of fibres from compound eyes composed of two nasal or two temporal half-fields. In this interesting experiment it appeared as if the recipient tectal half-field had spread to cover the entire tectum, producing a rather radical change in the pattern of tectal differentiation. However, as we suggested to Dr. Székely in a seminar at the Massachusetts Institute

of Technology a few years ago (see also Sperry, 1965), one should probably reserve judgment on the interpretation of these results until the effects of tectal hyperplasia and hypoplasia, in response to the overload and diminished innervation, can be ruled out. The spread of the innervated half of the tectal gradient into the denervated half may be more apparent than real and more mechanical than chemical. Related problems are mainly of embryological interest. They bear primarily on the developmental dynamics of the morphogenetic field and have only remote significance for problems of brain function.

A question with more direct neurological implications concerns the degree of local resolution and overlap in the optic fibre projection on the tectum. How large is the terminal arborization field of a single fibre and with how many tectal cells over how large a dendritic field does one optic fibre synapse? How much overlap is there in the synaptic zone of neighbouring individual fibres? How do these compare in the normal and in the regenerated condition? The answers to these and related questions may be expected to differ in different species and for different parts of the retina. At this stage a rough approximation would be helpful in judging the higher degrees of precision required in the regeneration process to restore normal vision. The maps based on electrical procedures are difficult to interpret in this connexion because of the variables associated with changes in amplification and the uncertainty whether the evoked potentials come from optic fibre terminals or from post-synaptic firing in the tectal neurons.

Using Golgi staining of the normal two-day-old chick tectum we have seen terminal arborizations of the optic fibres in considerable numbers, measuring about 75 μ or approximately 1/200th of the total arc of the central tectal field. A somewhat lower order of refinement is probably present in the cichlid fishes like the angel fish *Pterophyllum scalare* and *Astronotus ocellatus*, in which we have seen visual recovery with little if any loss in visual acuity. A large—22 per cent—loss of acuity on the average was reported in another study on *A. ocellatus* by Weiler (1966). The extent to which the regenerative process is able to reproduce the normal degree of resolution in visual function needs further study. It is difficult to judge the degree of selective precision of the growth of optic fibres from the pattern established normally because of the complex sequence of possible sustaining factors present in normal development. Observations on the interposition of obstacles and other types of interruptions in the fibre pattern within the tectum (see below) indicate that considerable correction is possible in regrowth within the plexiform layer close to the target zone.

Some years ago one of us (E. H.) noted a very striking similarity in the

layered differentiation of retina and tectum in the developing frog tadpole. This is illustrated in Fig. 1 for the ten-week-old tadpole of *Rana pipiens*. While the character of the layering and the thickness of the retina remains fairly uniform throughout, that of the tectum varies somewhat from the

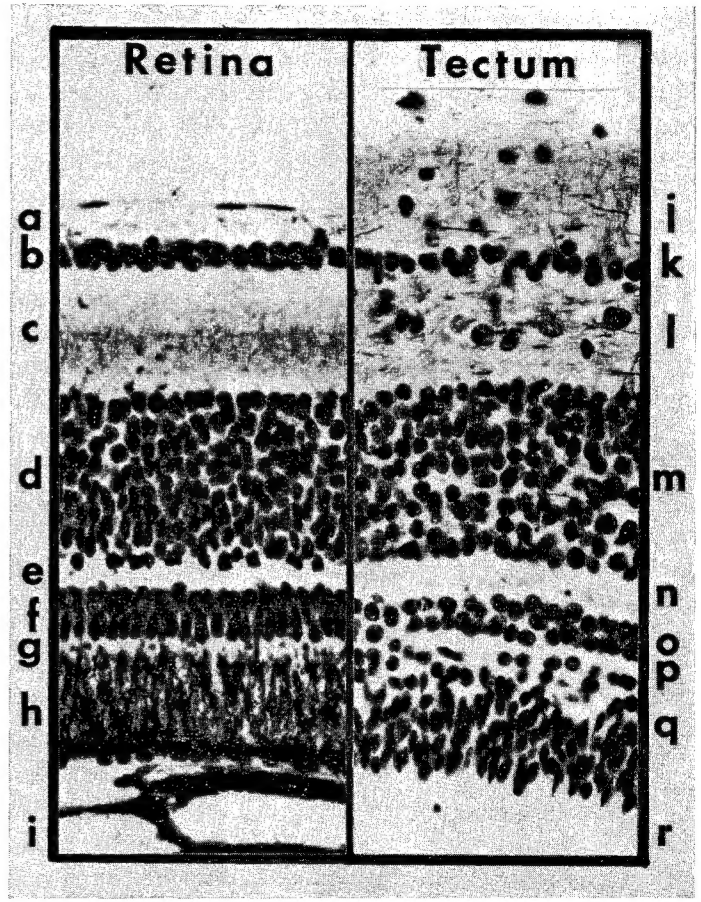

FIG. 1. Comparison of retinal and tectal stratification in a ten-week-old tadpole of *Rana pipiens*. × 400

(a) optic fibre layer
(b) ganglion cell layer
(c) inner plexiform layer
(d) inner nuclear layer
(e) outer plexiform layer
(f) outer nuclear layer
(g) limitans
(h) photoreceptor and pigment cell layers
(i) choroid vessels
(j) marginal and optic layers
(k) superficial grey layer
(l) deep medullary layer
(m) central grey layer
(n) deep white layer
(o) periventricular grey layer
(p) periventricular fibrous layer
(q) subependymal granular and ependymal layer
(r) ventricle

dorsomedial to the ventrolateral portions of the lobe, primarily in the thickness of the periventricular cell layer. The segment shown in Fig. 1 represents the central part of the lobe, corresponding approximately to the position where the centre of the visual field would be projected. The photographs are shown at identical magnifications, the distance from the innermost to the outermost uniform cell layers being 175 μ in both cases. The relative position of the optic nerve fibre layer is the same in both retina and tectum, but the thickness of the tectum is somewhat greater because of the marginal layer overlying the optic fibres. Although the number of cells in each layer arranged perpendicular to the surface is approximately the same (one in the ganglion cell and superficial grey matter, eight to ten in the inner nuclear and central grey matter, two in the outer nuclear and periventricular grey matter), the surface area of the tectum is less than 60 per cent of that of the retina. A considerable amount of convergence between retinal ganglion cells and terminal tectal sites seems indicated. The numbers and positions of cell layers, the distribution and thickness of plexiform layers, and the relative position of outgoing and incoming optic fibres in these structures appear to be more than coincidental.

We have been investigating in a preliminary way, with Dr. Arora (reviewed in Sperry, 1965), the forces that control the growth of optic fibres in various parts of the afferent course into the tectum, using surgical deflections, interruptions and transplantations. When the medial and lateral brachia of the optic tract are cut short and crossed at the anterior tectal pole the bulk of the sprouting fibres promptly turn back to regain their normal channel. When the lateral tract, cut long, is transposed deep into the medial pathway, most fibres enter the medial tract and follow it on across the equator of the tectum into the ventral quadrants before they enter the plexiform layer to synapse in their original zone. When contralateral fibres are superimposed on a fully innervated optic tract they form a plexiform zone only in the corresponding mirror locus of the opposite tectum.

The ability of the optic fibres to find their way to their proper tectal stations even from grossly abnormal starting positions has been investigated by surgical deflection of the cut optic nerve. The central end of the divided optic nerve was apposed to the root of the oculomotor nerve in such a way that the regenerating optic fibres then entered the brain below and caudal to the tectum, near the point where the oculomotor fibres leave. Gaze (1959) had previously reported a case in which the optic nerve inadvertently entered the brain along this route. He was able to record a few evoked potentials on the contralateral tectum corresponding to points of stimulation on the retina from which the nerve had originated, but was unable to

elicit optokinetic responses through this nerve. In the present series, the optic nerve was transposed caudally to a point near the roots of the oculomotor and trigeminal nerves in 14 *Xenopus laevis* tadpoles. In three cases, both optic nerves entered the brain through the oculomotor root, and most of the optic fibres succeeded in making their way back to the tectum.

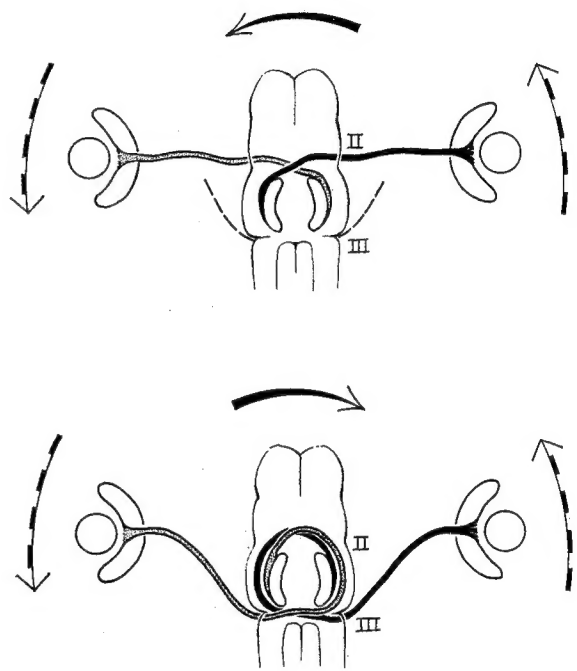

FIG. 2. Diagrams illustrating the courses taken by optic fibres from the eye to the tectum in normal *Xenopus* tadpole (upper), and tadpole in which the optic nerves have been diverted into the oculomotor nerve roots (lower). The response of the normal animal to counter-clockwise rotation of an optokinetic drum, indicated by banded arrows, is to turn to the left. The response of the experimental animal, in which optic fibres cross twice and terminate in the ipsilateral tectal lobes, is to turn in the opposite direction.

However, these connexions were made with the ipsilateral instead of the contralateral tectum—not by following the oculomotor fibres back towards the nucleus of cranial nerve III as might have been expected, but rather by decussating twice, once at the level of the IIIrd root and again in the region of the optic chiasma (see Fig. 2). Behaviourally, this led to reversed optokinetic responses in both directions in two animals, and spontaneous circling to the right in the third (Hibbard, 1967).

In two other cases, the left optic nerve regenerated over the surface of the brain above the meninges. In one of these latter, the aberrant optic nerve was cut again above the midbrain and poked down through a hole into the dorsal surface of the ipsilateral tectum. Thirteen days later this animal began to show spontaneous circling to the left at a rate of about 12 turns per minute. The deflected optic nerves in seven other cases strayed

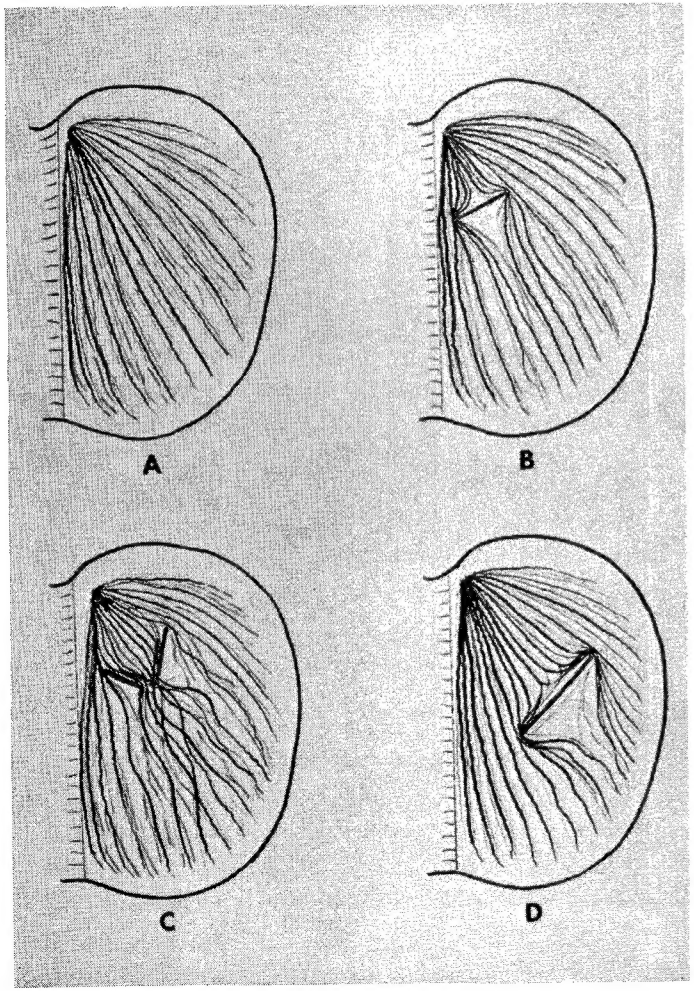

FIG. 3. Routes taken by regenerating optic fibres from the medial tract in the tectum when blocked by tantalum foil barriers. (A) Normal. (B) Small obstacle with fibre-free shadow behind it. (C) Two barriers through which fibres are funnelled. (D) Large barrier with fine fibres in the shadow area behind it.

caudally and entered the brain along the trigeminal nerve root. In traversing the ophthalmic ramus, ganglion, and root of the Vth nerve the optic fibres in these cases remained fasciculated and did not intermingle with trigeminal fibres. The optic fibres could not be followed any distance into the brain within the trigeminal tract and there was no evidence of visual recovery under these conditions.

The orderly, near-parallel distribution of optic fibres in the "parallel" or "radiation" layer of the tectum has yet to be explained. What are the invisible guidelines or forces that keep the fibres from crossing and interspersing and from curling up or dipping down? Are the invisible guidelines primarily mechanical or chemical or a combination of both? In an effort to get some answers to such questions we have made various kinds of experimental interruptions and derangements of the parallel layer in adult fishes, some examples of which are shown in Fig. 3. This work is still in progress, but a few points seem to be emerging. Although the deflected fibres seem not to be able to effect much change of course within the parallel layer itself, the same fibres may show extensive readjustment in the plexiform layer just below. This would appear to permit a considerable degree of target correction near the terminations and to reduce the degree of precision required at choice points in more distal portions of the optic fibre channels. The deflected fascicles undulate through the plexiform layer in smooth curves and gradually straighten out. We have not been able to substantiate Leghissa's (1955) description of a criss-cross overlap among the optic fibres of the parallel layer at the posterior equator.

If, after the optic fibres in the parallel fascicles have been cut, barriers of tantalum foil are inserted across their course, the regenerating fibres pass around the block in a smooth flow, leaving a "shadow" area in the parallel layer relatively clear of fibres immediately behind the block. If two strips of foil are placed at angles of 45° to the parallel bundles in such a way as to form a funnel through which the regenerating fibres will pass, the deflected fibres pass through the narrow opening, at angles of 90° and less, later crossing and re-crossing other fibres largely below or sometimes above the parallel layer proper. They gradually refill the parallel fascicles distal to the block, but whether the fibres are regaining the same channels as they would normally occupy or are merely refilling any empty pathways is not clear. The undulating pattern suggests an inertial component in the fibre growth, with oscillations and corrections of deviations from course by negative feedback.

Correction of the course of the outgrowing axon, apparently in response to an anteroposterior gradient effect, has been clearly shown in abnormally

positioned Mauthner cell axons (Hibbard, 1965). If the segment of hindbrain containing the Mauthner cell is reversed at an embryonic stage before the outgrowth of the axon, the axon first grows forward, towards the original posterior end of the grafted segment, but then re-curves to proceed down the length of the spinal cord. In embryos joined back-to-back near the end of the hindbrain (see Fig. 4) the Mauthner axon proceeds along its typical course into the twin, but travelling in the caudo-rostral direction directly opposite to normal. Presumably the microfilament flare at the growing tip does not span sufficient rostrocaudal differences to

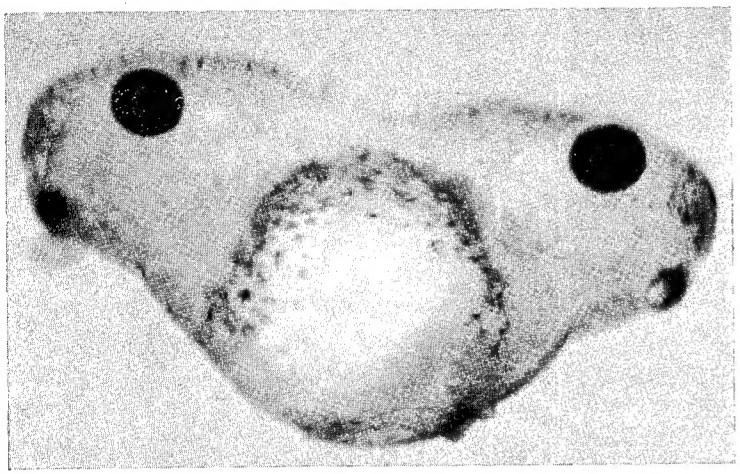

FIG. 4. Two-headed *Xenopus* embryo formed by joining anterior halves of two embryos to study the effect on the course of Mauthner's axons.

overcome the inertial growth component (Swisher and Hibbard, 1967). The direction of advance of a single growing fibre is probably better viewed as a reflection of the algebraic sum of the combined adhesive forces of all the active microfilaments than as the sequence of single strong filament successes. Van der Loos (1965), studying abnormally oriented pyramidal cells in the cortex, has suggested that the initial outgrowth of the axon, in some instances at least, as well as the spread of the dendrites, is intrinsically determined within the cell and is dependent upon the position of the cell, while further outgrowth of the axon is determined by extrinsic factors.

SUMMARY

The nerve tract connecting eye to brain, together with its afferent and efferent association within the retina at one end and within the midbrain tectum at the other, may be regarded as a model system for the study of the developmental organization of brain pathways and synaptic connexions. The physiological and behavioural properties of this system indicate a high degree of complexity, precision, diversity and refinement in the underlying neural relations; any understanding of the regulative factors by which this optic system is organized in growth should therefore take us a long way towards a general understanding of the basic developmental mechanisms for the inherent patterning of brain pathways and connexions.

The orderly formation of retino-tectal connexions, both in development and in regeneration, has been studied extensively in experiments involving surgical, behavioural, histological, electrical, biochemical and related methods, applied separately and in combination. The principal regulative factors disclosed so far by the accumulated evidence have been outlined and some of the behavioural and histological results briefly illustrated. Some newer observations have been presented that deal with the patterning of fibre routes and connexions within the tectum, with a correspondence in retinal and tectal stratification, with the growth patterns of transposed optic nerves, and with the oriented advance of individual axons. An attempt is made to show how the developmental mechanisms inferred from experiments on the retino-tectal relations of lower vertebrates could also account in principle for the ontogenetic organization of the much more complicated mammalian visual system.

Acknowledgements

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